PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P63765PC00	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/NL2004/000462	International filing date *(day/month/year)* 30.06.2004	Priority date *(day/month/year)* 30.06.2003
International Patent Classification (IPC) or national classification and IPC G01N1/30, G01N1/31, G01N1/36, B01J3/00		
Applicant ACADEMISCH ZIEKENHUIS GRONINGEN et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 2 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
24.03.2005	03.06.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Hocquet, A Telephone No. +31 70 340-2928

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/NL2004/000462

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-22	as originally filed
Claims, Numbers	
1-11	received on 24.03.2005 with letter of 23.03.2005
Drawings, Sheets	
1/7-7/7	as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☒ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☒ the claims, Nos. 12
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-11
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-11
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-11
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The following documents are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: EP-A-0 822 403 (MILESTONE S R L) 4 February 1998. D1 describes a clearing process during the preparation of tissues before embedding. The clearing is done at a high pressure (up to 10 bars) and high temperature (preferably 80 to 85 Celsius), the pressure being built by carbon dioxide in a closed container (D1, col. 4, lines 18-33). Under these conditions CO2 is not in a supercritical state (Tc=31°C, Pc=73 bars) nor in a near supercritical state as defined in the application (page 6, lines 15-18). Furthermore D1 discloses embedding using a pressure of more than 1 bar (D1, c 5, l 26-34).

D2: FRAYSSINET P ET AL: "Histological integration of allogeneic cancellous bone tissue treated by supercritical CO2 implanted in sheep bones" BIOMATERIALS, ELSEVIER SCIENCE PUBLISHERS BV., BARKING, GB, vol. 19, no. 24, December 1998 (1998-12), pages 2247-2253, XP004168858 ISSN: 0142-9612.
D4 discloses a method of analysis comprising contacting a biological sample (allogeneic bone) with a supercritical fluid for defatting it, followed (after a 1-8 months implantation) by an histologic analysis comprising embedding in PMMA.

D3: US 2003/072677 A1 (HOWANEC MYRON ET AL) 17 April 2003. D2 describes preparation of soft tissues for use as xenografts using supercritical fluid, and discloses also the use of compositions of supercritical fluids mixed with other processing agents used in the chemical treatment of tissues such as alcohols, or fixing agents.

D4: US-B1-6 493 964 (TOUSIMIS ANASTASIOS J ET AL) 17 December 2002. D3 discloses methods and devices for preparation of biological tissues for SEM using supercritical fluids.

D5: WO 01/44783 A (UNIV MIAMI ;ESSENFELD ERVIN (VE); ESSENFELD HAROLD (VE)) 21 June 2001 (2001-06-21)

1 The combination of the features of claim 1 is neither known from, nor rendered

obvious by, the available prior art: discloses many possible reasons are known for 'contacting a biological sample with a supercritical fluid' (see above) . But none of the available documents discloses a method where a supercritical fluid replaces the conventional xylene clearing for removing a dehydrating agent, the supercritical fluid being then replaced by infiltrating an embedding medium.

2 The combination of the features of claim 8 is neither known from, nor rendered obvious by, the available prior art: none of the available documents discloses a processor for preparing samples for histological analyses comprising the pressurizing and heating means for bringing a substance in supercritical phase, supplying means for supplying that substance to the reactor and supplying means for adding the embedding medium to the reactor.

3 Claims 2-7 and 9-11 meet the requirements of the PCT in respect of inventive step, because they depend respectively of claim 1 and 8.

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(44)

Amended Claims with letter of 23 March 2005

1. A method for processing a biological sample for histological analysis, comprising the steps of:
   a) contacting the sample with a dehydrating agent;
   b) removing the dehydrating agent with a composition comprising a supercritical or a near supercritical fluid at a temperature in the range of 0.7 to 1.4 times its critical temperature and at a pressure in the range of 0.3 to 7 times its critical pressure; and
   c) replacing the supercritical fluid by infiltrating an embedding medium, preferably paraffin, at a pressure of at least 1 bar.

2. A method according to claim 1, wherein said supercritical or near supercritical fluid is carbon dioxide.

3. A method according to claim 1 or 2, wherein said biological sample is a fresh, frozen or fixed tissue sample, preferably a fresh, non-fixed sample.

4. A method according to any one of claims 1-3, wherein said biological sample comprises an organ or a part thereof.

5. A method according to any one of claims 1-4, wherein said sample is dehydrated, defatted and/or decalcified prior to impregnation by using a composition comprising a supercritical fluid.

6. A method according to claim 5, wherein said composition additionally comprises a dehydrating agent, preferably an alcohol.

7. A method according to claim 5 or 6, wherein said composition additionally comprises a decalcifying agent, preferably an acid.

8. A processor (1) for preparing at least one sample (10) for histological analysis, comprising at least one process reactor (9) for the at least one sample (10), characterized in that the processor (1) comprises supplying means (4) for supplying to the reactor (9) at least one substance of which at least one is in supercritical phase or near supercritical phase and at least one supplying means (7) for adding the embedding medium to the reactor (9) through conduit (8) and further comprises pressurizing and/or heating means (5, 6) for bringing a substance at the required pressure and/or temperature.

9. A processor (1) according to claim 8 , further comprising separating means (11) for separating substances from a mixture of substances leaving the reactor (9).

10. A processor (1) according to claim 8 or 9, further comprising recycling means (13) for recycling substances discharged from the reactor (9).

11. Use of a processor according to any one of the claims 8 to 10 for processing a biological sample for histological analysis.